CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

4-(2-NITROBUTYL) MORPHOLINE AND 4,4'-(2-ETHYL-2-NITROTRIMETHYLENE) DIMORPHOLINE

Chemical Code # 002113 and 2114, Tolerance # 50239
SB 950 # 769
July 31, 2003, revised September 3, 2004

I. DATA GAP STATUS

Chronic toxicity, rat:	Data gap, no study submitted.[1]
Subchronic rat, dermal	No data gap, possible adverse skin effects
Chronic toxicity, dog:	Data gap, no study submitted[1]
Oncogenicity, rat:	Data gap, no study submitted[1]
Oncogenicity, mouse:	Data gap, no study submitted[1]
Reproduction, rat:	Data gap, no study submitted[1].
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	Data gap, no study submitted[1].
Gene mutation:	No data gap, possible adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 212998 were examined.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: T040903
Original: Kishiyama and Gee, 7/31/03, revised by Gee, 9/3/04
These two chemicals are used in combination as antimicrobials for fuels, metal working cutting fluids and other industrial uses. There is no US EPA RED at this time.

[1] These studies are not required for a non-food use antimicrobial at this time.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

No Study Submitted.

CHRONIC TOXICITY, RAT

No Study Submitted.

CHRONIC TOXICITY, DOG

No Study Submitted.

ONCOGENICITY, RAT

No Study Submitted.

ONCOGENICITY, MOUSE

No Study Submitted.

REPRODUCTION, RAT

No Study Submitted.

TERATOLOGY, RAT

** 50239 - 010 114630 Griffin, T. B. "A Teratology Study of Bioban P-1487 in Rats." (Coulston International, Inc., White Sands Research Center, Laboratory Project ID 890402, October 22, 1990.) Bioban P-1487 (lot 8K15-DF28, 84%) was administered via gavage at doses of 0, 10, 30, or 100 mg/kg/day during gestation days 6 through 15 to 25 mated Sprague-Dawley female rats/group. Body weight, body weight change and food consumption were lower for mid-dose and, particularly, the high-dose groups. Clinical signs were seen at the mid and high doses, consisting of excessive salivation, fur staining and "red" urine, especially at 100 mg/kg/day. Maternal NOEL = 10 mg/kg/day. There were no fetal malformations or other treatment-related statistically significant effects. There was slight increase in the percent postimplantation loss (29% versus 21%) at the high dose versus controls and 5/25 total litter resoprtions at the high dose

compared with 1/25 controls (p = 0.95) but these were not statistically significant. Nominal developmental NOEL = 100 mg/kg/day. UNACCEPTABLE. Upgradeable (analysis of dosing material). (Kishiyama and Gee, 7/31/03).
The three following records were submitted to address the deficiencies noted above. The cover letter states that dosing analyses were not performed at the time the study was conducted, the conducting laboratory no longer is in business and the records for weighing of the active ingredient for dose preparation cannot be located by the present registrant. Record 212997 addresses the issue of stability. Unfortunately, none of the above records can satisfy the question of the actual content of the test solutions. The data from the study indicated a dose response for maternal findings, so that there must have been an increase in the actual doses, reflecting the nominal doses. Given the lack of any developmental toxicity in the absence of maternal toxicity, it is unlikely that a repeat of the study would result in any additional findings. The deficiency of the actual doses remains. A retrospective study, one method of addressing the deficiency, would not satisfy the deficiency because the laboratory no longer exists and the records of dosing preparation cannot be located. At the time the risk assessment is conducted, it may be deemed appropriate to add an additional uncertainty factor, based on the lack of dosing analysis data. The study is considered adequate for filling the data gap, given the above caveat. (Gee, 9/3/04)

50239 - 024 212996 Amendment No. 1 to the protocol, signed on July 12, 1989, describing how the dosing solutions would be prepared.

50239 - 024 212997 Report of the stability of 4-(2-nitrobutyl)-morpholine over 6 months. The report was prepared by Case Consulting Laboratories, Whippany, NJ, dated November 11, 2002. The initial content of 4-(2-nitrobutyl)-morpholine was 76% and that of Bioban P-1487, 83.63 as area %, for lot OE1531LAN4. After 6 months at room temperature (temperature not given), the area % was 89.96. This stability obviously refers to a much later lot of Bioban P-1487 but, since the test solutions in the teratology study were prepared weekly, there is no reason to question stability during the study.

50239 - 024 212998 This consists of a report on the characterization of Bioban P-1487, lot OJ04311LAN7, dated March 6, 2002. The purity of this lot was determined to be 86.3%. Impurities were also identified

TERATOLOGY, RABBIT

No Study Submitted.

GENE MUTATION

** 50239 - 011 114631 Lynn, S. P. "Ames Mutagenicity Assay Bioban P-1487^{R}." (Toxikon Corp., Project Number 90G-0526. August 2, 1990.) Bioban P-1487^{R} (lot 8K15-DF28, purity 96%) was assayed at concentrations of 0.007, 0.29, 0.12, 0.47, 1.88 and 7.5 μg/plate, with and without rat liver metabolic activation, for mutagenicity using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537. There were triplicate plates per concentration in a single trial. Positive controls were functional. Bioban P-1487 was toxic at 7.5 ug/plate with fewer spontaneous revertants. There was no significant increase in revertants with Bioban P-1487

treatment. No adverse effect. ACCEPTABLE. (Kishiyama and Gee, 7/31/03)

**** 50239 - 0020 211438** Linscombe, V. A., M. R. Schisler and K. F. Treadway " Evaluation of Bioban ™ P-1487 in the mouse lymphoma (L5178Y TK +/-) forward mutation assay." (Toxicology & Environmental Research and Consulting, Dow Chemical Company, Midland, MI, Laboratory ID 011110, April 4, 2002) Bioban™ P-1487 (4-(2-nitrobutyl)morpholine (80.86%) and 4,4'-(2-ethyl-2-nitromethylene)dimorpholine (4.12%)) was assayed with mouse lymphoma L5178Y cells for forward mutation. Two independent assays were conducted, both with and without rat liver S9 activation. Concentrations yielding a relative total growth of $\leq$ 10% were not evaluated for mutants. Concentrations evaluated were as follows: Assay B1, no activation: 0, 1.25 and 2.5 ug/ml - higher concentrations were cytotoxic. Assay B1, with activation: 0, 1.25, 2.5, 5.0 and 7.5 ug/ml. Assay C1, no activation: 0, 0.25, 0.5, 1, 2, 3 and 4 ug/ml. Assay C2, with activation: 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 9 ug/ml. There was no evidence of a significant increase in mutants without activation. The study was considered to present evidence of a positive response in the presence of activation in both assays. A total mutant index of 3.3 at 7.5 ug/ml in assay B1 and of 4.81 at 9 ug/ml, 3.62 at 8 ug/ml and 3.11 at 7 ug/ml in Assay C2 were reported. Possible adverse effect. ACCEPTABLE. (Gee, 8/17/04)

CHROMOSOME EFFECTS

** 50239 - 0021 211439 Spencer, P. J. " Evaluation of Bioban P-1487™ in the mouse bone marrow micronucleus test." (Toxicology & Environmental Research and Consulting, Dow, Midland, MI, study 020146, March 14, 2003) CD-1 male mice (6/group for controls, low and mid doses and 10 for the high dose) were dosed with Bioban P-1487™ at 0 (corn oil), 125, 250 or 500 mg/kg/day for two consecutive days and sacrificed for evaluation 24 hours after the second dose. Dose selection and justification for using only male mice in the definitive study were based on two range-finding studies. In the range-finding studies, four mice/sex were given doses of 0, 500, 1000 or 2000 mg/kg body weight on two consecutive days. Animals were observed for at least 72 hours until day 4 with day 1 the first day of dosing. A second set of mice, 4/sex/dose, were given doses of 0, 125 or 250 mg/kg/day, due to high mortality in the first set. All mice died at 1000 and 2000 mg/kg/day. All control and 500 mg/kg animals survived. Clinical signs at 500 mg/kg included decreased activity, periocular and perineal staining. Body temperature was also was affected (decreased) at 1000 and 2000 mg/kg. In the second set of animals, all animals survived until scheduled sacrifice. At 250 mg/kg, periocular staining was noted. In the definitive study, 3/10 males died at 500 mg/kg before scheduled sacrifice. Also, 9/10 showed decreased activity. There was no increase in micronucleated polychromatic erythrocytes. The % PCEs at 500 mg/kg/day was statistically significantly lower than controls. The positive control, 120 mg/kg cyclophosphamide, was functional. No adverse effect. ACCEPTABLE. (Gee, 9/1/04)

50239 - 011 114632 Bollmeier, A. F. and L. S. Desai. “Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells.” (Toxikon Corp., Project Number: 89G-0095, June 29, 1989.) Bioban P-1487 (lot 8K15-DF28, purity 85% by wt.) was evaluated for genotoxicity at concentrations of $1x10^{-8}$ and $5 x10^{-8}$ to $1x10^{-5}$ and $5 x10^{-5}$ ml/ml with and without rat liver metabolic activation using Chinese hamster ovary cells. The method of dilution of the liquid technical material to achieve the concentrations noted in the tables was not explained. There were duplicate cultures per concentration with fifty cells scored per slide. Exposure time was 8 hours without activation followed by three additional hours with colcemid. With activation, exposure

was for 2 hours followed by 8 additional hours of incubation with colcemid added the final 3 hours. Cells were collected by mitotic shakeoff. Cytotoxicity data were reported only for the preliminary test where exposure was for 8 hours without activation followed by a 24 hour expression period and the number of cells were counted. The report implies the same test was performed with activation but no data were included. In the definitive test, there was no significant increase in the percent of cells with aberrations or number of chromosomal aberrations per cell. Cytotoxicity for the definitive test was not reported other than "mortality" at the highest concentration of $1x10^{-5}$ ml/ml medium without S9 and $5x10^{-5}$ with activation. No individual culture data were reported and the types of aberrations were not described. Also, the positive control data for EMS without activation and for cyclophosphamid with activation were identical. UNACCEPTABLE (no individual data, method of dilution of test material not described and the data for the positive controls should be confirmed). Upgradability uncertain. (Kishiyama and Gee, 7/31/03)

50239 - 023 212407 A copy of the Data Evaluation Record by US EPA for record 114632, in which their classification was "provisionally acceptable." See R040903 for a discussion of this submission. No change in the status of record 114632 as a result of this additional submission. (Gee, 9/3/04)

DNA DAMAGE

** 50239 - 0019 211437 Cifone, M. A. " *In vivo/in vitro* unscheduled DNA synthesis in rat primary hepatocyte cultures at two timepoints with a dose rangefinding assay with Bioban™ P-1487." (Covance Laboratories, VA, Study No. 23633-0-494, Dow No. 021036, August 25, 2002) Bioban™ P-1487 (86.3% 4-(2-nitrobutyl)morpholine and 0.64% 4,4'-(2-ethyl-2-nitromethylene) dimorpholine) was given to male Fischer 344 rats by oral gavage in a single dose of 0 (corn oil), 450 or 900 mg/kg. Groups of 5 - 7 per group were evaluated at 2 - 4 hours and 14 - 16 hours after dosing for UDS in isolated hepatocytes, using autoradiography. Dose selection and justification for a single sex were based on rangefinding studies in which 3/sex were given single doses of 0, 300, 450, 600, 750 or 900 mg/kg with a repeat with male rats at 900 mg/kg. Animals were observed for 2 days following dosing and clinical signs recorded at 0.5, 2-4 hours and 1 and 2 days postdose. All females given 750 and 900 mg/kg were found dead by day 2 but all males survived at these doses. Males at 600 mg/kg and above showed clinical signs which included urine stains, red crust/stains in several locations, and few feces. Based on the rangefinding study, only males were used and the dose of 900 mg/kg selected as the high dose. Hepatocytes were isolated from perfused livers, placed into cultures (3 for UDS and 1 for attachment efficiency per animal), labeled for 4 hours with ^{3}H-thymidine followed by a 16 - 20 hour incubation with unlabelled thymidine. Viability was evaluated by trypan blue dye exclusion for the perfusate and for attachment viability. The net nuclear grain counts from 50 cells per culture were scored for a total of 150 per animal. The positive control, dimethylnitrosamine (10 and 15 mg/kg), was functional. There was not evidence of unscheduled DNA synthesis at either dose given *in vivo*. ACCEPTABLE. (Gee, 9/2/04)

50239 - 011 114633 Bollmeier, A. F. and L. S. Desai. “Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures.” (Toxikon Corp., Project Number: 89G-0094, August 3, 1989.) Bioban P1487^{R} (lot 8K15-DF28, 85% by weight) was assayed for the induction of repair of DNA damage at concentrations of 5×10^{-5}, 1×10^{-5}, 5×10^{-6}, 1×10^{-6}, $5 \times 10^{-7}$and 1×10^{-7} ml/ml using

primary rat hepatocytes. There were triplicate coverslips with fifty cells evaluated on each per concentration. The protocol for the UDS is unclear in terms of the time of treatment versus exposure to tritiated thymidine for labelling. This needs clarification. A preliminary cytotoxicity assay was performed using trypan blue dye exclusion. According to the report, the primary hepatocytes used in the UDS assay were held overnight while the cytotoxicity assay was run, not the usual protocol. No viability data were included in the report in terms of initial viability, attachment viability or control viability over time. No increase in net nuclear grains was reported at any concentration but only summary data were presented. UNACCEPTABLE (protocol unclear, viability data not included, nuclear and cytoplasmic grain counts not reported, individual culture results not reported). Upgrade uncertain. (Kishiyama and Gee, 7/31/03).

50239 - 023 212406 A copy of the Data Evaluation Record of US EPA for record 114633 in which the classification was "acceptable." The deficiencies noted in the original review of record 114633 still exist and there is no change in status. See R040903 for further discussion.

OTHER

Note: 50239 - 022, 212308, contains the requested information on determination of the volumes to be used per animal. The collective data from the two studies is acceptable for a dermal subchronic study with a systemic NOEL = 300 mg/kg/day and a dermal NOEL < 30 mg/kg/day, based on skin irritation. (Gee, 9/3/04)

** 50239 - 009 114628 Griffin, T. B. “Subchronic 90-Day Dermal Toxicity Study of Bioban P-1487 in Rats.” (Coulston International, Inc., White Sands Research Center, Laboratory Project ID 89020, June 5, 1990.) Bioban P-1487 (lot 8K15-DF28, 84% if the two major components) was administered dermally at doses of 0, 30, 100, or 300 mg/kg/day, 6 hours/day, 5 days/week for 90 days to 20 Sprague-Dawley rats/sex/group. Bioban P-1487 was applied undiluted - volume calculations were not described. Test article was spread over one quarter of the shaved area with site rotation weekly. The site was covered with gauze and tape. Treatment caused generally similar incidences of irritation (erythema and scaly appearance) of the skin at all doses with none in the water control groups. No other toxicologically significant effects were reported. Results indicated the high dosage may have been too low for dermal route - see record 117394. UNACCEPTABLE but possibly upgradeable (description of dose determination) (Kishiyama and Gee, 7/29/03). See note above. (Gee, 9/3/04)

** 50239 - 012 117394 Griffin, T. B. “Subchronic 90-Day Toxicity Study of Bioban P-1487 in Rats (Limit Test).” (Coulston Research, Inc., Laboratory Project ID 910302, July 31, 1992.) Bioban P-148 (lot 8K15-DF28, purity 85% by wt.) was evaluated for dermal toxicity at 0 (water) and 1000 mg/kg (Limit test), applied 5days/week for 13 weeks to 20 Sprague-Dawley rats/sex/dose. Two treated females were sacrificed on days 22 and 26 due to poor condition, possibly related to treatment. There were no toxicologically significant effects on hematology, clinical chemistry or ophthalmology. Body weight and weight gain were reduced in both sexes at 1000 mg/kg/day. There was no consistent effect on food consumption. Organ weights: relative liver weight was increased in treated females. Chronic inflammation of the urinary bladder occurred in 10 males and 12 females compared with 1 in each control group, cause unknown. Bioban P-1487 caused skin irritation at the treatment site, primarily acanthosis, with a higher incidence in females. NOEL <1000 mg/kg/day (body weight, skin irritation).

UNACCEPTABLE (method for determining dosing volume was not described) Upgradeable. This study should be considered in conjunction with record 114628. (Kishiyama and Gee, 7/30/03). See note above. (Gee, 9/3/04)